OPTIMIZATION AND PRODUCTION OF ALGINATE LYASE USING MARINE BACTERIA AND ITS APPLICATION IN BIOFILM CONTROL

A SYNOPSIS

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1. INTRODUCTION

Alginate lyases are enzymes that degrade alginate by a β-elimination reaction through cleavage of the 1– 4 O-linkage between the uronic acids in the linear polymer [1]. In recent years, the production of alginate lyase using microorganisms has gained much attention due to its wide range of applications. Alginate lyases are useful in therapy for cystic fibrosis by enhancing the antibiotic effect on mucoid *Pseudomonas aeruginosa* [2]. Alginate lyases also find applications as biocatalysts in the processing of renewable biochemicals and biofuels [3]. The use of alginate lyase to control alginate fouling has been reported recently. Although alginate lyase has several attractive applications, its high production cost and low productivity restrict the use of these molecules commercially. This issue could be overcome by the use of a higher yielding strain, process with low capital and operating cost, optimizing process parameters and media with the use of cheap substrates for production, controlled systems, and culturing strategies with minimal or manageable by-products. Seaweeds can serve as the most promising biomass for the production of biochemicals. The adequate disposal of seaweed wastes coupled with enzyme production is needed. From the literature review, it was understood that despite having a wide range of potential applications, the alginate lyase enzyme is currently not in commercial use and alginate lyase production has not been reported using solid substrate fermentation.

In this perspective, the present study was focused on screening, isolation, and characterization of alginate-degrading bacteria from different sponges and seaweeds collected from Tuticorin, Gulf of Mannar, Tamilnadu, India. The marine bacteria was identified as *Enterobacter tabaci* RAUC using biochemical analysis and 16S rRNA sequencing. Further, the production of alginate lyase using this bacterial isolate was carried out using submerged and solid substrate fermentation. The process parameters and media were optimized for enhanced production of alginate lyase. Statistical design of experiments along with ANFIS modeling approaches were employed for optimization of production media. Brown seaweed, *Sargassum swartzii* biomass was used as a low-cost substrate for the production of alginate lyase using solid-state fermentation and the process was optimized in this study.

Further, the crude *Enterobacter tabaci* RAU2C alginate lyase was immobilized using a bacterial exopolysaccharide, curdlan gum. The process of immobilization of

enzyme onto the curdlan matrix was optimized. The soluble and immobilized enzyme was characterized in terms of optimum pH, temperature and substrate concentration. The kinetic parameters of both forms of enzymes were evaluated.

Then, the potential of alginate lyase as an anti-biofilm candidate against the *P. aeruginosa* biofilm was also assessed. Further, *in silico* characterization of reported alginate lyases enhanced the understanding of structural features which are crucial for enzyme design and engineering. The variability among alginate lyase sequences and physiochemical properties offers avenues for tailored enzyme development to suit specific industrial and therapeutic needs.

Overall, the comprehensive exploration of alginate lyase from isolation to application underscores their significance in various fields, promising further advancements in biotechnology and healthcare.

2. LITERATURE REVIEW

2.1 Alginate

As per Zhu et al., alginate is a naturally occurring anionic polysaccharide constituting 40% of the dry weight of brown seaweeds and is the main structural element of these algae [4]. Additionally, Wong et al. (2000) noted that besides being a primary structural component of brown seaweeds, alginate is also produced by bacterial species such as *Pseudomonas* and *Azotobacter* [5]. Alginate is an unbranched copolymer composed of β-D-mannuronate (M) and α-L-guluronate (G) units. These molecules exist in three different kinds of blocks: poly α-L-guluronate (polyG), poly β-D-mannuronate (polyM), and a heteropolymer (polyMG). Smidsrod et al reported that owing to abundant marine biomass and its low cost, alginate has found applications in various sectors, including textile, food, cosmetic, and pharmaceutical industries. The composition of alginate can vary depending on factors such as polymer length, the relative amount and distribution of G residues, and the acetyl content. All of these factors are controlled by alginate-modifying enzymes. Alginate is a versatile ingredient that finds application in various industries including the food industry. It is widely used in the food industry as a thickening, gelling, and stabilizing agent. It is particularly renowned for its ability to encapsulate flavors and create gel-like textures, making it a popular choice in dairy products, desserts, sauces, and bakery items. Alginate's gel-

forming properties make it a valuable ingredient in pharmaceutical formulations. It can be used to create controlled-release systems for drug delivery, ensuring that medications are released at a desired rate [1].

2.2 Alginate Lyases

Smidsrod et al. (1966) outlined that alginate lyases, also referred to as alginate depolymerases or alginases, are enzymes responsible for cleaving alginate. These lyases can break 1 → 4 glycosidic bonds in alginate through the β-elimination process. Based on their substrate specificity, alginate lyases are categorized into three groups: polyG lyases (EC 4.2.2.11), polyM lyases (EC 4.2.2.3), and polyMG lyases (EC 4.2.2.-). The specificity of the substrate is often determined by the environment in which the lyase-producing organism is found, and it can be adapted to the type of alginate that is readily accessible [1]. Based on the mode of action, alginate lyase can be classified into endolytic and exolytic alginate lyase. Kim et al. (2011) elucidated that endolytic alginate lyases degrade alginate by cleaving glycosidic bonds, yielding unsaturated oligosaccharides such as disaccharides, trisaccharides, and tetrasaccharides. Conversely, exolytic alginate lyases degrade oligosaccharides into monosaccharides [6].

2.3 Sources of Alginate Lyases

Wong et al reported that alginate lyases are produced by a diverse array of organisms, including marine and soil bacteria. Additionally, these enzymes have been identified in marine algae, marine mollusks, fungi, bacteriophages, and viruses [5]. Alginate lyase has been reported in various microbes, including Li et al in *Pseudoalteromonas sp* [3], Hutcheson et al in *Saccharophagus degradans* [7], Yamasaki et al in *Sphingomonas sp* [8], Ochiai et al in *Agrobacterium tumefaciens* [9], Swift et al in *Microbulbifer* mangrove [10], Sawant et al in *Bacillus cereus* [11], Nibu et al in *Enterobacter cloacae* [12], Ertesvåg et al in *Azotobacter vinelandii* [13], Lange et al in *Klebsiella aerogenes* [14], and Nguyen et al in *Streptomyces sp* [15].

2.4 Applications of Alginate Lyase:

In agriculture, pharmaceuticals, and the food industry, Zhu et al and Rocha & Santos highlighted the diverse applications of alginate lyase. This enzyme is instrumental in

both the production of oligosaccharides from alginate and the elucidation of alginate's structure [4, 16]. In the utilization of alginate lyases, Inoue et al illustrate their wide application in the production of red and brown algal protoplasts [17], while Aarstad et al delved into their role in elucidating the fine structure of alginate [18]. Additionally, Li et al highlighted their involvement in synthesizing alginate oligosaccharides [3]. These oligosaccharides, as discussed by Courtois et al exhibit intriguing biological actions. Ranging from two to twenty-five monomers, alginate oligosaccharides have been implicated in various biological functions and find utility in the agriculture, food, and pharmaceutical industries [19]. Notably, Reddy et al attributed to alginate oligosaccharides anti-inflammatory, antioxidant, anti-bacterial, and prebiotic activities [20]. Kawada et al and Iwamoto et al demonstrated that alginate oligosaccharides promote the secretion of cytotoxic cytokines by human macrophages and the development of human endothelial cells. Because of their ability to act as biocatalysts for the synthesis of functional oligosaccharides and ethanol, alginate lyases have garnered a lot of interest [21, 22]. Islan et al highlighted the exceptional promise of alginate lyase in the management of cystic fibrosis by dissolving bacterial polysaccharide biofilms [23]. Alkawash et al suggested its potential use as therapeutics to enhance the effectiveness of antibiotics for *Pseudomonas aeruginosa* mucoid therapy [24].

2.5 Optimization and Production of Alginate Lyases

The optimization and production of alginate lyases are important for maximizing their efficiency and yield in industrial applications. Different strategies can be employed to optimize and produce alginate lyases. Factors such as carbon and nitrogen sources, metal ions, incubation temperature, inoculum concentration, starting pH, agitation/shaking, and incubation period are the primary determinants of a microorganism’s ability to produce enzymes for industrial use. Improving the enzyme yield is heavily dependent on optimizing these parameters. Since this is the case, optimizing the media components, culture factors, and fermentation conditions is crucial for producing industrial enzymes in sufficient quantities. In most cases, one process factor is optimized at a time while the others remain constant, and the next round of trials takes the optimized condition or component into account in sequential sequence. An economical fermentation method may be designed with the use of

optimized nutritional parameters, physicochemical features, and fermentation variables. To speed up the manufacturing of industrial enzymes, statistical approaches are also utilized to optimize the process parameters.

Beltagy et al reported that *Pseudomonas stutzeri* showed higher alginate lyase activity at pH 7.5 [24]. Further, Nibu et al reported that the alginate lyase production using *Enterobacter cloacae* was higher at pH 7.8 [12]. Ma et al reported maximum alginate lyase production with *Pseudoalteromonas sp*. after 24 hours with 3% inoculum [25]. The highest production yield of alginate lyase by *Pseudoalteromonas tetraodonis* was achieved in a medium with the following composition (g/L): sodium alginate 5, ammonium dihydrogen phosphate 11.6, sodium chloride 32.9, ferrous sulfate 0.03, magnesium sulfate 0.08, and dipotassium hydrogen phosphate 0.4. This optimization was accomplished using surface response methodology and central composite design (CCD), as outlined by Tang et al. in 2013 [26]. Fu et al established that the optimal conditions for maximizing alginate lyase production by marine *Vibrio sp.* YKW-34 included an inoculum volume of 10% and an inoculum age of 12 hours. Moreover, they determined that the ideal temperature for alginate lyase production was 25°C. The fermentation medium comprised 0.5% *Laminaria* powder and 0.2% KNO_3, with an initial pH of 8.0 [27].

2.6 Statistical Design of Experiments and Soft Computing Approaches in Optimization

Phan-Tan-Luu & Cela emphasized the pivotal role of the statistical design of experiments in research and data analysis. This structured approach efficiently gathers data, analyzes relationships between variables, and facilitates drawing informed conclusions [28]. Steinberg & Hunter accentuated that through systematic investigation of the effects of multiple factors on response variables, researchers can discern key drivers and relationships within complex systems. This process enhances the validity and reliability of results [29]. Bezerra et al and Wijngaard and Brunton highlighted the fundamental role of methods such as the Plackett–Burman design and Response Surface Methodology (RSM). These methods have been instrumental in efficiently exploring relationships between variables and optimizing experimental outcomes [30, 31]. Gan and Latif explained that the Plackett–Burman screening approach facilitates the identification of significant variables that influence the response. Conversely,

Response Surface Methodology (RSM) enables a comprehensive quantification of parameter-response relationships [32]. Ganesan et al. highlighted that the integration of the Adaptive Neuro-Fuzzy Interface System (ANFIS) utilizes fuzzy logic and artificial neural networks to forecast optimal responses in nonlinear processes [33].

Myers highlights Response Surface Methodology (RSM) as a powerful statistical and numerical optimization technique, particularly in industries such as the chemical and process industries. This methodology enables the empirical fitting of computed responses as a function of input parameters, thereby facilitating the optimization of processes and product designs. Notably, RSM has found application in optimizing medium composition, parameters in oil extraction, and various other processes, offering enhanced efficiency compared to traditional methods. RSM's evolution includes advancements in generalized linear models and considerations for design robustness, expanding its applicability beyond conventional boundaries [34]. Furthermore, Loganathan and Girija noted that the Adaptive Network-based Fuzzy Inference System (ANFIS) represents a hybrid soft computing approach that combines Artificial Neural Network (ANN) and Fuzzy Inference System (FIS) methodologies. This integration effectively addresses challenges encountered in statistical optimization processes [35]. Additionally, Takagi and Sugeno have also emphasized the effectiveness of ANFIS in this regard. ANFIS's integration of backpropagation and least squares algorithms enables effective modeling of both linear and nonlinear input and output variables, further enhancing its utility in data-driven decision-making processes.

2.7 Seaweeds as a Low-Cost Substrate for Enzyme Production

Seaweed biomass has garnered significant attention across various industries due to its abundance, rapid growth rate, and diverse biochemical composition. Nigam highlights that in bioenergy, seaweed serves as a promising feedstock for biofuel production, including bioethanol, biodiesel, and biogas [37].
Its carbohydrate-rich nature, particularly polysaccharides, as highlighted by Roesijadi et al makes seaweed suitable for fermentation processes to produce bioethanol [38]. Additionally, Mata et al. demonstrated that its lipids can be converted into biodiesel through transesterification processes [39]. Moreover, Horn & Horn illustrated that anaerobic digestion of seaweed biomass can generate biogas, providing a renewable

energy source [40]. In agriculture and aquaculture, the nutrient-rich composition of seaweed biomass, as noted by Khan et al and Wong et al enhances soil fertility, promotes plant growth, and improves crop yields [41, 42]. Overall, seaweed biomass represents a versatile and sustainable substrate with significant potential for applications in bioenergy production, agriculture, and bioremediation, contributing to environmental sustainability and resource efficiency.

Seaweed biomass finds utility as a substrate in solid-state fermentation for enzyme production. Solid-State Fermentation (SSF) or Solid-State Bioconversion (SSB), as elucidated by Pandey enables more efficient production of enzymes and metabolites while consuming less energy [43]. Panesar et al highlighted that owing to its advantages such as simplicity, ease of product extraction, high yield, and production of concentrated products, Solid-State Fermentation (SSF) can serve as an effective technology for the production of enzymes and a viable alternative for the production of value-added products from seaweed biomass [44]. Rodríguez-Jasso et al used algal biomass as substrate for fungal fucoidanase production in a rotating drum bioreactor using SSF [45]. Wang et al developed an alternative economical process for reducing alginate content in the *L. japonica* feed through semi-solid fermentation with alginate-degrading *B. amyloliquefaciens* WB1 [46]. Pervez et al reported the utilization of algal biomass such as *Dictyopteris polypodioides*, *Sargassum wightii*, *Dictyopteris divaricata*, *Ulva lactuca* and *Codium tomentosum* for the production of pectinases using SSF. Among all, *U. lactuca* was reported to be the best substrate for pectinase production using *Bacillus licheniformis* KIBGE-IB4 and a higher yield of the enzyme was reported when compared to the usage of agricultural biomass as substrate [47]. Fernandes et al reported the sequential bioprocessing of *Ulva rigida* to synthesize lignocellulolytic enzymes, thereby expanding its potential as aquaculture feed in terms of nutritional merit [48]. Additionally, Lara et al achieved cellulase production using *Cladosporium sphaerospermum* through Solid-State Fermentation (SSF) with green macroalgae *Ulva fasciata* as a substrate [49].

2.8 Immobilization of Enzymes

Ji et al highlighted that the major challenges in utilizing enzymes are their potential instability and limited reuse. To address this issue, one approach is the immobilization

of enzymes, which involves fixing or attaching them to a solid support or matrix. This immobilization technique can enhance enzyme stability, increase their longevity, and improve their operational performance. Some of the techniques include gel encapsulation, retention on transducers using semi-permeable membranes, surface immobilization through adsorption or covalent binding, and nanomaterial-based immobilization. These immobilization techniques provide a means to enhance the stability of alginate lyase, improve its reusability, and optimize its performance in industrial applications. In addition to the methods mentioned earlier, surface immobilization techniques have been explored for alginate lyase and other enzymes. These techniques involve attaching the enzyme to a solid support, such as adsorption onto solid surfaces or covalent binding. The use of surface immobilization techniques, such as adsorption or covalent binding, allows for the attachment of alginate lyase and other enzymes onto a solid support, which enhances their stability and facilitates their recovery from the reaction mixture. This immobilization method also provides better control over the catalytic activity of alginate lyase and enables its reuse in industrial processes [50].

2.8.1 Curdlan as a Support Matrix:

One potential support matrix for enzyme immobilization is curdlan gum, a microbial polysaccharide. Curdlan has several properties that make it suitable for immobilizing enzymes. Jiménez et al outline several advantages of curdlan. Firstly, they highlight its biocompatibility and biodegradability, rendering it environmentally friendly. Secondly, its unique three-dimensional structure with a rigid helical conformation provides a stable microenvironment for enzyme immobilization and helps maintain the enzyme's structural integrity. Furthermore, curdlan's high surface area and porosity enable increased enzyme loading and efficient substrate diffusion. Lastly, the authors note that curdlan can be easily modified or functionalized to enhance its compatibility with specific enzymes and improve the immobilization process [51].

2.9 Potential of Alginate Lyase as an Anti-biofilm Candidate

Bacterial biofilms are complex structures formed by communities of bacteria that adhere to surfaces and are embedded within a self-produced extracellular matrix. Cai et al discuss various strategies researchers have been investigating to prevent the

formation of biofilms and disrupt existing ones. One such strategy involves using enzymes as an anti-biofilm agent. Alginate lyase specifically targets and degrades the alginate polymer, a major component of the extracellular matrix in Pseudomonal biofilms. By breaking down the alginate matrix, alginate lyase can weaken the structural integrity of biofilms and enhance the efficacy of anti-bacterial treatments [52]. This approach holds promise for developing new therapeutics to combat biofilm-related infections and prevent their recurrence. Furthermore, the potential for alginate lyase to disrupt biofilms and enhance antimicrobial effects in medical applications is a promising area for further investigation.

Mrsny et al and Murata demonstrated that treatment with alginate lyase has been shown to decrease viscosity in clinical cultures and sputum of cystic fibrosis patients [53, 54]. Lamppa et al and Strathmann et al showed their effectiveness in eliminating biofilms from abiotic surfaces [55, 56]. Additionally, Eftekhar and Mai et al found that it improves phagocytosis and killing of *P. aeruginosa* by human immune cells [57, 58]. Furthermore, Alkawash et al highlighted its role in enhancing the therapeutic effectiveness of several anti-pseudomonal antibiotics [2]. Considering these facts, alginate lyase can be utilized for the treatment of cystic fibrosis through the depolymerization of alginate-rich biofilm of mucoid *P. aeruginosa*. Mahajan et al. (2021) reported that the endotypic alginate lyases CaAly and VspAlyVI, the exotypic alginate lyases FspAlyFRB and SA1-IV, and the glycoside hydrolase PslG were found to significantly inhibit the formation of biofilms in *P. aeruginosa* [59].

3. OBJECTIVES

1. To isolate, screen, and characterize potent alginate lyase-producing bacteria from the marine environment.
2. To optimize the bioprocess conditions and medium composition for maximal production of alginate lyase through submerged fermentation.
3. To check the feasibility of using seaweed as a low-cost substrate for alginate lyase production through solid-state fermentation.
4. To immobilize the alginate lyase enzyme using curdlan gum and to characterize the soluble and immobilized alginate lyase produced from marine bacteria.

5. To study the application of *Enterobacter tabaci* RAU alginate lyase as an anti-biofilm agent against *P. aeruginosa.*
6. *In silico* analysis of alginate lyases reported in the literature.

4. RESEARCH PROBLEM

Although alginate lyase has several attractive applications and demand, its high production cost and low productivity restrict the use of these molecules commercially. Therefore, exploration of novel high-yielding strains, optimizing process parameters and media with the use of low-cost substrates for production, controlled systems, and culturing strategies with minimal or manageable by-products can be possible solutions to address this issue and promote the commercial employment of these enzymes. Firstly, while several bacteria are known to be potential producers of alginate lyase, there remains a need for further exploration and identification of novel strains with even higher yields and efficiency. Additionally, the optimization of production processes, including media composition, cultivation conditions, and scale-up strategies, could significantly enhance the commercial viability of alginate lyase production. Moreover, despite the demonstrated efficacy of alginate lyase in biofilm disruption, there is still a lack of understanding regarding the mechanistic details of its action on biofilms, as well as its potential applications in other medical and industrial contexts. Furthermore, the *in-silico* characterization of alginate lyase highlights the variability among different strains, suggesting the need for a deeper understanding of structure-function relationships to optimize enzyme properties for specific applications. Finally, while alginate lyase immobilization shows promise for enhancing stability and reusability, further research is needed to explore better immobilization methods through novel matrices and the impact of immobilization on enzyme kinetics and performance. Addressing these research gaps would not only advance our fundamental understanding of alginate lyase but also unlock its full potential for various practical applications in biomedical, food industry, and bioenergy.

5. ORGANIZATION OF THE THESIS

This thesis consists of nine chapters, which are arranged in the following structure.

Chapter 1: Introduction

Chapter 1 delves into a comprehensive introduction to the study and the significance of the research work. The chapter highlights the objectives of the research work.

Chapter 2: Literature review

Chapter 2 of the literature review delves deeply into the intricate world of alginate and alginate lyases. The exploration begins with a comprehensive examination of alginate and alginate lyase, highlighting their importance and relevance in various fields. The chapter progresses to elucidate the structure, classification, mechanism of action, and sources of alginate lyase, providing a solid foundation for further understanding. The chapter sheds light on the diverse applications of alginate lyase, showcasing its versatility and potential impact across industries. Furthermore, the chapter reviews the optimization and production of alginate lyase reported in the literature, emphasizing the significance of the statistical design of experiments and ANFIS modeling in experimental research design for maximizing efficiency and efficacy. The chapter reviews the advantages of enzyme immobilization and the utilization of curdlan gum as an immobilization agent. The potential of alginate lyase as an anti-biofilm agent was also reviewed.

Chapter 3: Isolation, screening, and characterization of potent alginate lyase-producing bacteria from the marine environment

In Chapter 3, the intricate process of screening, isolating, and characterizing alginate lyase-producing bacteria from the marine environment is meticulously detailed. Beginning with the collection of sponges and seaweeds from the Gulf of Mannar, the chapter outlines the methodical steps taken to transport and enrich these samples in the laboratory environment. Utilizing an enrichment method, bacteria capable of producing alginate lyase are isolated and subsequently screened for their enzymatic activity. This screening involves primary assays to detect activity, followed by secondary assays for quantification, providing a comprehensive evaluation of each isolate's potential. Furthermore, the chapter delves into the characterization process, which includes microscopic and biochemical tests, as well as ribosomal RNA sequencing to identify and confirm the marine bacterial isolate. By employing

advanced techniques such as 16S rRNA gene sequencing and subsequent BLAST analysis, the isolated marine bacteria were identified as *E. tabaci* RAU2C.

Chapter 4: Optimization of bioprocess conditions and medium composition for alginate lyase production

Chapter 4 focuses on the optimization of alginate lyase production using *E. tabaci* RAU2C. The optimization process involves fine-tuning various parameters to enhance the yield of alginate lyase through submerged culture methods. Initially, pH optimization was conducted to determine the optimal pH range for enzyme production. Following this, nitrogen and mineral sources are screened using the Plackett-Burman design to identify the most influential components. Subsequently, optimization of media composition was performed using Response Surface Methodology (RSM) and Central Composite Design (CCD) to determine the optimal levels of significant factors for maximum enzyme production. The chapter also explores the use of the Adaptive Neuro-Fuzzy Inference System (ANFIS) to further optimize media constituents. Experimental verification was carried out to validate the accuracy of the optimized models. Additionally, optimization of production time, inoculum size, and inoculum age were investigated to maximize alginate lyase production by the marine bacteria. The optimization results revealed a 16-fold increase in enzyme activity, as depicted in Figure 1. This significant enhancement underscores the effectiveness of the optimization process in augmenting enzyme performance.

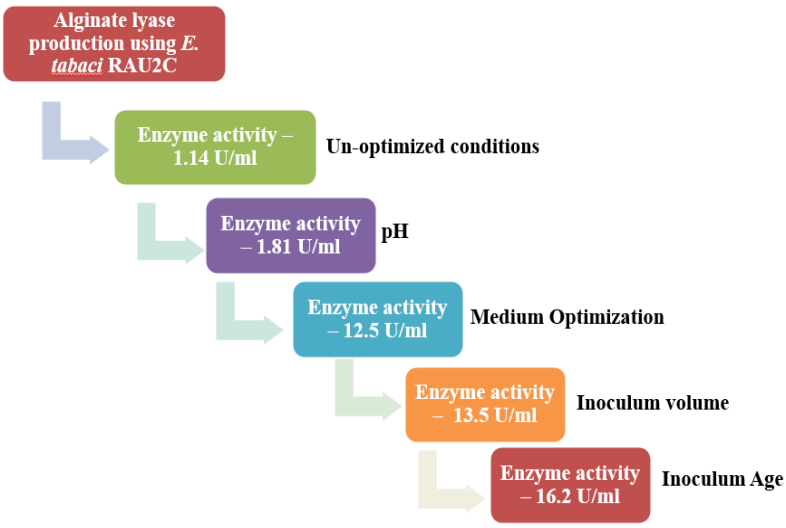


Figure 1: Summary of Optimization Studies

Chapter 5: Solid-state fermentation of brown seaweeds for the production of alginate lyase using marine bacterium

Chapter 5 of the research delves into the collection and preparation of seaweed biomass feedstock, along with the characterization of the seaweed biomass and the production of alginate lyase enzyme through solid-state fermentation (SSF). Brown seaweed, *Sargassum swartzii*, collected from the Gulf of Mannar was employed as a substrate for *E. tabaci* RAU2C alginate lyase production. This chapter deals with the optimization of process parameters such as incubation time and moisture content for maximal production of alginate lyase. Furthermore, the potential application of SSF waste residue as a biofertilizer for promoting plant growth using *Spinacia oleracea* as a model system was also investigated and discussed in this chapter.

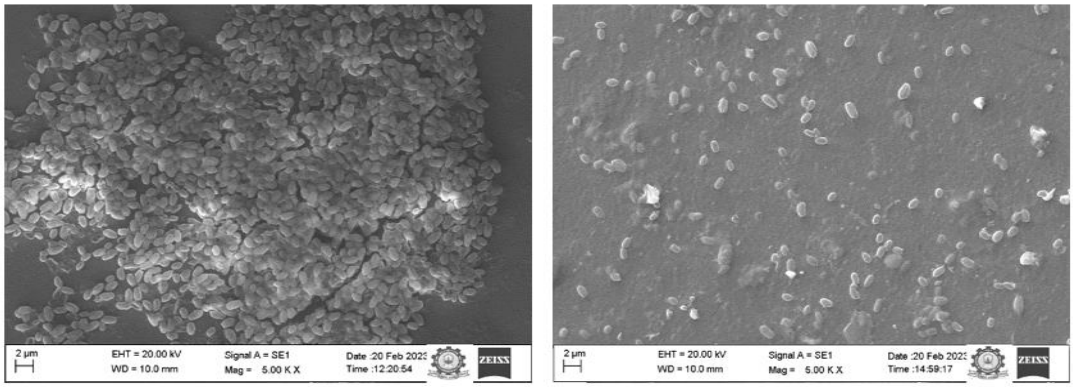
Chapter 6: Immobilization of alginate lyase enzyme using curdlan gum and characterization of the soluble and immobilized alginate lyase

Chapter 6 discusses the immobilization of *E. tabaci* RAU2C alginate lyase using bacterial exopolysaccharide curdlan gum. The method for optimal immobilization of the crude enzyme was assessed. The immobilization efficiency and reusability of immobilized alginate lyase were studied and discussed in this chapter. The effects of temperature, pH, and substrate concentration on soluble and immobilized alginate lyase were examined. The kinetic parameters of the immobilized enzyme were determined indicating improved catalytic efficiency compared to the soluble form.

Chapter 7: Evaluation of Anti-*Pseudomonas aeruginosa* biofilm activity of alginate lyase of *Enterobacter tabaci* RAU2C

Chapter 7 investigates the anti-biofilm activity of alginate lyase produced from *E. tabaci* RAU2C against *P. aeruginosa* biofilm. The study includes biofilm inhibition and disruption assays using a crystal violet microtiter plate method. Results indicate that crude *E. tabaci* RAU2C alginate lyase inhibits biofilm formation in a dose-dependent manner, with significant inhibition observed at higher enzyme concentrations. Furthermore, the enzyme effectively disrupts pre-existing biofilms as investigated by microscopic analysis as shown in Figure 2. The optimal time for enzyme treatment was also studied and discussed in this chapter. Overall, the findings suggest that *E. tabaci* RAU2C alginate lyase has promising potential as an anti-biofilm

agent against *P. aeruginosa*, highlighting its application in therapy and preventing and eliminating biofilms in medical devices.



(a) (b)

Figure 2: SEM image of *P. aeruginosa* biofilm-(a) control (b) after treatment with *Enterobacter tabaci* RAU2C alginate lyase

Chapter 8: Computational analysis of alginate lyases produced by different microorganisms

Chapter 8 of the thesis focuses on the computational analysis of alginate lyases using various tools and methods. Alginate lyase sequences were retrieved from the UniProt database and subjected to multiple sequence alignment, revealing conserved domains among sequences from different microorganisms. Phylogenetic analysis highlighted evolutionary relationships among the sequences, while physicochemical analysis provided insights into their properties. Motif analysis identified conserved motifs shared among alginate lyases, aiding in functional characterization. Homology modeling was used to generate 3D structures of the enzymes and, the active sites were predicted using computational tools. Overall, the study offers a comprehensive characterization of various alginate lyases, providing valuable information for enzyme design and engineering.

Chapter 9: Summary and Conclusion

Chapter 9 encompasses a summary and conclusion which includes a multifaceted exploration of alginate lyase production, optimization, characterization, application, and computational analysis. Beginning with meticulous screening, isolation, and characterization of alginate lyase-producing bacteria from marine sources, the

research delves into the optimization of production methods using *E. tabaci* RAU2C. It further investigates the utilization of seaweed biomass for enzyme production through solid-state fermentation, alongside the immobilization of alginate lyase for potential industrial applications. The study also elucidates the anti-biofilm activity of alginate lyase against *P. aeruginosa* biofilms, highlighting its therapeutic potential. Additionally, computational analysis sheds light on the structural and functional characteristics of alginate lyases, offering insights for enzyme engineering. Collectively, these findings contribute to a comprehensive understanding of alginate lyases, their production, optimization, and diverse applications, paving the way for further advancement in the field.

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7. LIST OF PUBLICATIONS

1. **Ramya, P**., Selvaraj, K., Suthendran, K. et al. (2023) Optimization of alginate lyase production using *Enterobacter tabaci* RAU2C isolated from marine environment by RSM and ANFIS modelling. Aquaculture International 31, 3207–3237.
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3. **Ramya, P**., Aanandhalakshmi, R.., & Vanavil, B. (2023) Computational Analysis of Alginate Lyases Produced by Different Microorganisms. Current Trends in Biotechnology and Pharmacy, 17(3B), 1191–1207.
4. **P. Ramya**, P. Ezhilarasi, R. Aanandhalakshmi, P. Bazeera Ferdhous, and B. Vanavil, (2023) Oligosaccharides as Nutraceuticals In: The Nature of Nutraceuticals: History, Properties, Sources, and Nanotechnology, Apple Academic Press (Book Chapter- In Press).
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